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# Design, synthesis and pharmacological screening of 4-amino-5-pyrimidinecarbonitriles as potential anti-inflammatory agents

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**ABSTRACT**

*The present investigation is concerned with the synthesis of different diaryl substituted 4-amino-5- pyrimidinecarbonitriles with the objective of discovering novel and potent anti-inflammatory agents. Structures of the synthesized compounds were elucidated by spectral analyses. The obtained compounds were evaluated for their anti-inflammatory activities as well as gastric ulcerogenic effects. Results showed that 4c and 4h showed potent anti-inflammatory activity in carrageenan-induced rat paw edema test with low gastric ulcerogenicity compared with etoricoxib.*

**Key words:** anti-inflammatory, pyrimidine, COX-2.

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## INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are an unhomogeneous family of pharmacologically active compounds used in the treatment of acute and chronic inflammation, pain, and fever. However, nevertheless NSAIDs are the most widely used drugs, their long-term clinical employment is associated with significant side effects and the steady use determines the onset of gastrointestinal lesions, bleeding, and nephrotoxicity.[1-2] Therefore the discovery of

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new safer anti-inflammatory drugs represents a challenging goal for such a research area. Although several mediators support the inflammatory processes, the main target of NSAIDs is cyclooxygenase (COX),[3] the enzyme involved in the first step of the conversion of arachidonic acid to prostaglandins (PGs).These latter regulate important functions in the gastric, renal and ematic systems and are known to mediate all inflammatory responses. Classical NSAIDs, such as indomethacin, inhibit both isoforms of COX[4] -COX-1, which is constitutively expressed in most tissues and organs and catalyzes the synthesis of PGs involved in the regulation of physiological cellular activities; COX-2,which is mainly induced by several stimuli such as cytokines, mitogens and endotoxins in inflammatory sites.[5] Thus, their therapeutical effects are mainly due to the decrease of proinflammatory PGs produced by COX-2, whereas their unwanted side effects result from the inhibition of constitutive COX-1 isoform. Recently highly selective COX-2 inhibitors belonging to the classes of diarylheterocycles and methanesulfonanilides have been developed and marketed.

Furthermore, it had been reported that many compounds having a diaryl pyrimidine skeleton possessed significant anti-inflammatory activity.[6-8]

**Figure 1 Pharmacophore with 2 (blue) and the modeled structure A (red) of phenylsulphonyl tricyclic series fitted**
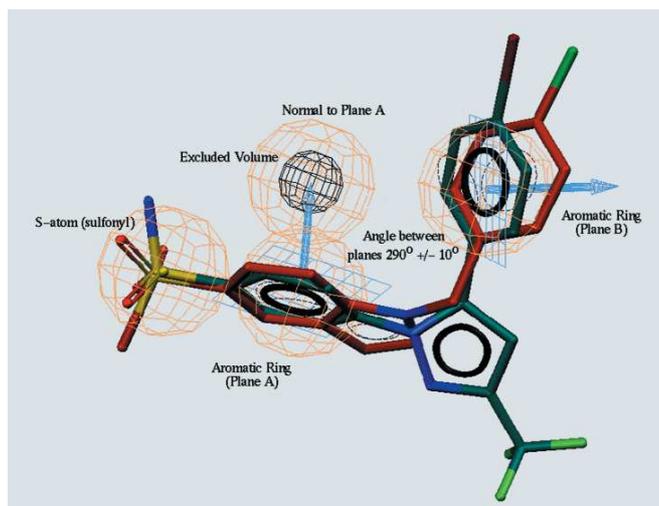


Basic pharmacophoric requirement for binding with COX-2 enzyme are (figure 1) two suitably substituted aryl rings on the adjacent atoms of the five membered or six membered ring system.[9] Etoricoxib is recently introduced COX-2 inhibitor which has six membered heterocycle (pyridine) as centered ring.

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Etoricoxib                              Targeted compounds
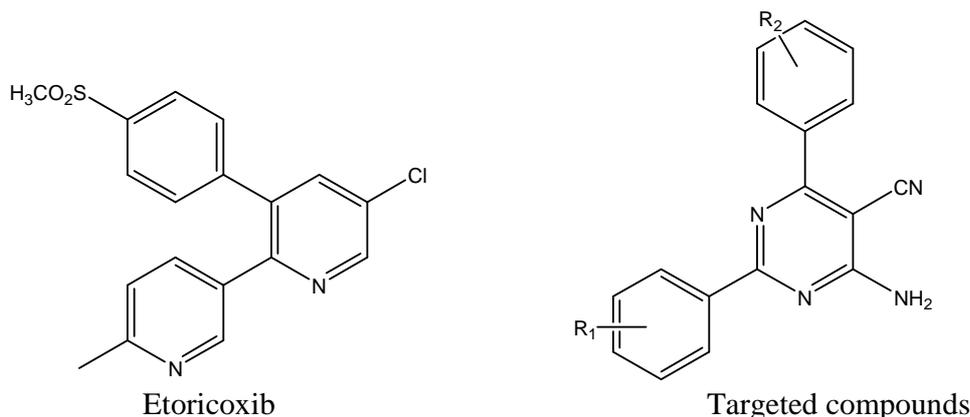
Based on pharmacophoric requirement, it was thought of interest to study the effect of 2, 6-diaryl substituent on central pyrimidine ring for anti-inflammatory activity. Based on this we have designed series of 4-Amino-6-*(un)*substitutedlphenyl-2-*(un)*substitutedphenyl-5-pyrimidinecarbonitrile as a potential anti-inflammatory agents.

## EXPERIMENTAL SECTION

Melting points of all compounds were determined in open capillaries and were uncorrected. IR spectra were recorded on a FTIR-8400 spectrophotometer (Shimadzu, Japan), using DRS prob. ^{1}H NMR (300 MHz) spectra were carried out on Varian EM-360L, 300 MHz, using $DMSO_{d6}$ as a solvent and the chemical shifts are given in δ (ppm). All NH and $NH_2$ protons were exchangeable with $D_2O$. Mass spectra were determined using direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu, Japan). All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 $GF_{245}$ precoated sheets 20-20 cm, layer thickness 0.2 mm (E-Merck) and were visualized by UV-lamp at wave length (l) 254 nm. All chemicals and solvents were of reagent grade and the latter were distilled and dried before use.

### Synthesis of benzamidine hydrochloride dihydrate from benzonitrile[10]

Dry HCl gas was passed into a cooled solution of benzonitrile (51 ml, 0.5 mol) in 32 ml of absolute alcohol until 31.3 g gas was absorbed. The reaction mixture was allowed to stand for 6 h. The solid cake of imidoether HCl was quickly crushed in a dry mortar. The solid was transferred in a flask containing 150 ml absolute alcohol and passed dry ammonia gas in this reaction mixture till 12 g of ammonia gas was absorbed by reaction mixture. The reaction mixture was shaken for 24 h and then allowed to stand for 48 h and filtered to remove the ammonium chloride precipitates. The filtrate was allowed to evaporate to dryness in air and the benzamidine hydrochloride thus obtained was dissolved in water. The solution was acidified with 25 ml concentrated HCl and then filtered. The filtrate was kept in refrigerator for 24 h. Crystalline benzamidine hydrochloride dihydrate were filtered and dried.

### General procedure for the preparation of 4-amino-5- pyrimidinecarbonitriles (4a-j)

A mixture of aldehyde (2 mmol), malononitrile (2 mmol), amidine hydrochloride (2 mmol) and sodium acetate (2 mmol) in $H_2O$ (50 mL) and ethanol (5 mL) was refluxed with stirring for 6 hours (the progress of the reaction being monitored by TLC and using n-hexane/ethyl acetate as

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an eluent). The product precipitated from the reaction mixture after cooling, and the solid was filtered and recrystallized from ethanol.

*4-Amino-2,6-diphenyl-5-pyrimidinecarbonitrile (4a):* White crystals; mp 210-212 °C; IR (KBr) 3478, 3344 (NH$_2$), 2212 (CN), 1641, 1617 (C=N), 1542 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 7.52-8.41 (m, Ar and NH$_2$); MS, *m/z* (%): 272 (M$^+$, 100), 169 (87), 142 (32), 104 (57), 77 (59), 51 (35).

*4-amino-6-(4-chlorophenyl)-2-phenylpyrimidine-5-carbonitrile (4b):* White crystals; mp 218-222 °C; IR (KBr) 3460, 3334 (NH$_2$), 2190 (CN), 1632, 1608 (C=N), 1562 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 7.54-8.56 (m, Ar and NH$_2$); MS, *m/z* (%): 308 (M$^+$, 100), 306 (32)

*4-amino-2-phenyl-6-p-tolylpyrimidine-5-carbonitrile (4c):* White crystals; mp 208-210 °C; IR (KBr) 3456, 3321 (NH$_2$), 2208 (CN), 1651, 1623 (C=N), 1557 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 2.38 (3H, s, CH$_3$), 7.42-8.20 (m, Ar and NH$_2$); MS, *m/z* (%): 286 (M$^+$)

*4-amino-6-(4-methoxyphenyl)-2-phenylpyrimidine-5-carbonitrile (4d):* White crystals; mp 202-204 °C; IR, (KBr) 3428, 3364 (NH$_2$), 2234 (CN), 1632, 1618 (C=N), 1561 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 3.85 (3H, OCH$_3$), 7.52-8.41 (m, Ar and NH$_2$); MS, *m/z* (%): 302 (M$^+$)

*4-amino-6-(4-nitrophenyl)-2-phenylpyrimidine-5-carbonitrile (4e):* Yellow crystals; mp 194-196 °C; IR (KBr) 3438, 3323 (NH$_2$), 2198 (CN), 1634, 1607 (C=N), 1542 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 7.51-8.38 (m, Ar and NH$_2$); MS, *m/z* (%): 317 (M$^+$)

*4-amino-2-(4-chlorophenyl)-6-phenylpyrimidine-5-carbonitrile (4f):* White crystals; mp 202-204 °C; IR (KBr) 3433, 3324 (NH$_2$), 2202 (CN), 1645, 1609 (C=N), 1544 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 7.48-8.45 (m, Ar and NH$_2$); MS, *m/z* (%): 308 (M+2), 306 (M$^+$)

*4-amino-2,6-bis(4-chlorophenyl)pyrimidine-5-carbonitrile (4g):* White crystals; mp 216-220 °C; IR (KBr) 3467, 3334 (NH$_2$), 2210 (CN), 1640, 1606 (C=N), 1540 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 7.50-8.34 (m, Ar and NH$_2$); MS, *m/z* (%): 345 (M+4), 343 (M+2), 341 (M$^+$)

*4-amino-2-(4-chlorophenyl)-6-p-tolylpyrimidine-5-carbonitrile (4h):* White crystals; mp 204-208 °C; IR (KBr) 3467, 3332 (NH$_2$), 2202 (CN), 1631, 1603 (C=N), 1539 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 2.38 (3H, s, CH$_3$), 7.40-8.48 (m, Ar and NH$_2$); MS, *m/z* (%): 322 (M+2), 320 (M$^+$)

*4-amino-2-(4-chlorophenyl)-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile (4i):* White crystals; mp 232-234 °C; IR (KBr) 3456, 3323 (NH$_2$), 2209 (CN), 1641, 1614 (C=N), 1532 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 3.85 (3H, OCH$_3$), 7.76-8.57 (m, Ar and NH$_2$); MS, *m/z* (%): 338 (M+2, 32) 336 (M$^+$)

*4-amino-2-(4-chlorophenyl)-6-(4-nitrophenyl)pyrimidine-5-carbonitrile (4j).* yellow crystals; mp 198-200 °C; IR (KBr) 3478, 3334 (NH$_2$), 2234 (CN), 1638, 1609 (C=N), 1538 (Ar) cm$^{-1}$; $^1$H NMR (300 MHz, DMSO$_{d6}$) 7.67-8.47 (m, Ar and NH$_2$); MS, *m/z* (%): 353 (M+2), 351 (M$^+$).

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**Scheme 1: Synthesis of various 4-amino-5-pyrimidinecarbonitriles**
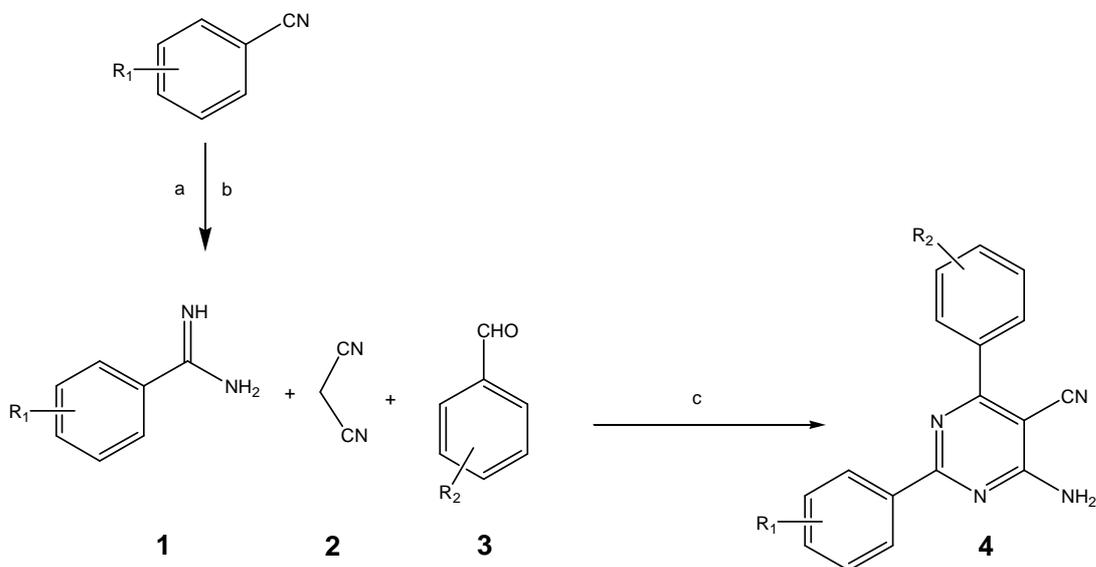


Reaction condition: a = ethanol, hydrogen chloride, b = amonia gas, c = sodium acetate, ethanol, water

| Comp. code | R$_1$ | R$_2$ | Comp. code | R$_1$ | R$_2$ |
|------------|-------|-------|------------|-------|-------|
| **4a** | H | H | **4f** | 4-Cl | H |
| **4b** | H | 4-Cl | **4g** | 4-Cl | 4-Cl |
| **4c** | H | 4-CH$_3$ | **4h** | 4-Cl | 4-CH$_3$ |
| **4d** | H | 4-OCH$_3$ | **4i** | 4-Cl | 4-OCH$_3$ |
| **4e** | H | 4-NO$_2$ | **4j** | 4-Cl | 4-NO$_2$ |

## Anti-inflammatory activity
### Animals
Albino wistar rats (300–350 g;) were housed in a controlled environment and provided with standard rodent chow and water. Animal care was in compliance with the CPCSEA regulations (rkcp/med/rp/10/05).

### Carrageenan-induced paw edema
Rats received a subplantar injection of 0.2 mL saline containing 2% γ-carrageenan in the right hind paw. A suspension of tested compounds (50 mg/kg) or an equivalent volume of vehicle, were administered intraperitoneally 30 min before carrageenan. Control animals received the same volume of vehicle. The volume of the paw was measured by plethysmometry immediately after the injection. Subsequent readings of the volume of the same paw were carried out after 1h and 3h and compared to the initial readings.[11] we used sigma statistical software for statistical evaluation of activity data.

## RESULTS AND DISCUSSION

We report the three-component reaction of aromatic aldehydes 1, malononitrile 2 and amidines 3 in water at reflux and in the presence of an equivalent amount of sodium acetate for 6-8 h,

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allowing the one-pot formation of 2-amino-5-pyrimidinecarbonitriles 4 in good yields (Scheme 1).[12]

In order to optimize the reaction conditions for preparing compounds **4**, the synthesis of 4-amino-2,6-diphenyl-5-pyrimidinecarbonitrile **4a** was carried out under different reaction conditions. Reaction of benzaldehyde **1a**, malononitrile **2** and benzamidine hydrochloride **3a** in DMSO at reflux in the presence of a catalytic amount of triethylamine was too slow and the yield was low, for example, even after 16 h compound **4a** was obtained in only 40% yield, as compared to yield of 78% in 6 h under thermal aqueous conditions.[13]

The complete process represents an example of a one-pot process with sequential steps (often referred to as tandem or cascade reaction) where reagents and catalysts are mixed together and experimental conditions are adjusted in such a way as to promote the reaction cascade. Thus the benzylidenemalononitrile **A** containing an electron-poor C=C double bond is produced by rapid Knoevenagel condensation of malononitrile with the aromatic aldehyde, the formation of the benzylidenemalononitrile being monitored by TLC (*n*-hexane/diethyl ether as eluent). The second step is followed by Michael addition, cycloaddition, isomerization, aromatization to afford the 4-amino-5-pyrimidinecarbonitriles **4**. Intermediate **B** is not stable and was not isolated from the reaction mixture. It must be easily oxidized by air to produce compound **4**. We believe the driving force for such a transformation is the aromaticity of these final products.

**Figure 2 Mechanism of reaction**
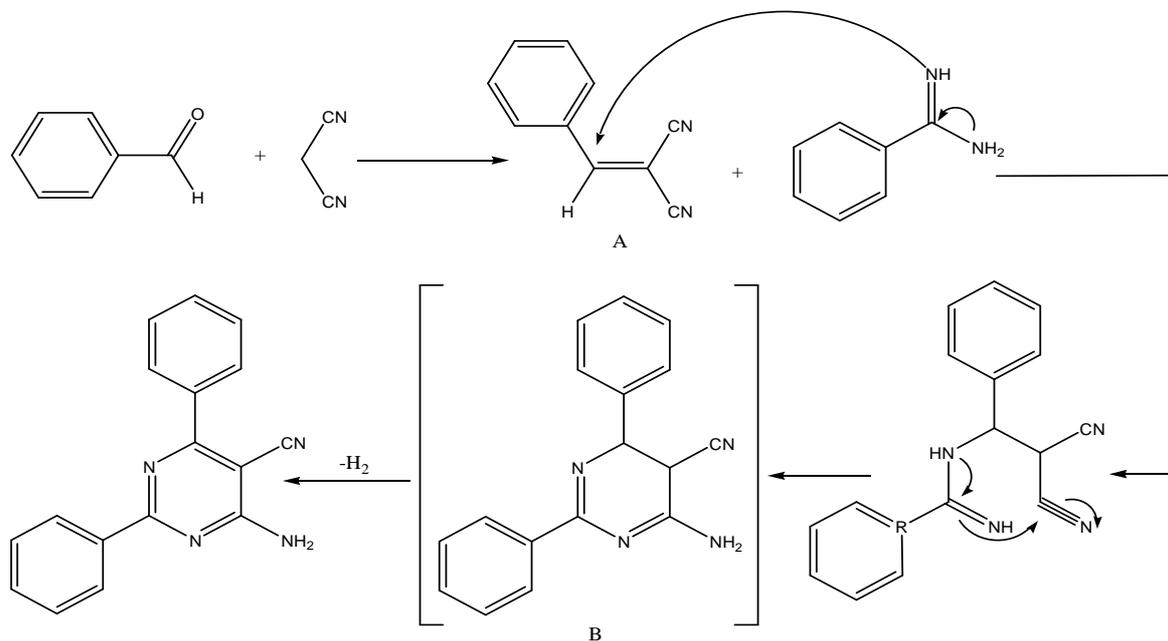


The use of water as solvent in organic chemistry has received increasing attention in the last decade. The enhanced reactivity and selectivity observed in some reactions have been rationalized by various authors as being a consequence of hydrophobic effects and enforced hydrophobic interactions. When the reaction was carried out in alcoholic solution good yields were obtained due to the solubility of all the reagents in alcohol solvent.

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The preliminary anti-inflammatory activity of the synthesized 4-amino-5-pyrimidinecarbonitriles derivatives were evaluated against carrageenan-induced rat paw edema. Results of the anti-inflammatory activity of the tested compounds as well as etoricoxib are shown in Table 1. Results showed that most of the tested compounds exhibited significant (P < 0.05) inhibition against carrageenan induced rat paw edema and comparable anti-inflammatory activity relative to etriocoxib. Among these derivatives, compounds 4c and 4h were found to be equally potent like etoricoxib.

**Table 1 Anti-inflammatory activity of 4-amino-5-pyrimidinecarbonitriles derivatives at 50 mg/kg dose level**

| Comp no. | $R_1$ | $R_2$ | % inhibition | |
|---|---|---|---|---|
| | | | 1h | 3h |
| 4a | H | H | $20.38 \pm 1.76$ | $21.69 \pm 1.54$*** |
| 4b | H | 4-Cl | $14.77 \pm 4.48$** | $18.04 \pm 4.16$* |
| 4c | H | 4-CH$_3$ | **51.88 ± 5.29*** | **66.26 ± 5.13*** |
| 4d | H | 4-OCH$_3$ | $31.48 \pm 3.22$ | $45.67 \pm 3.33$* |
| 4e | H | 4-NO$_2$ | $29.61 \pm 3.71$*** | $40.96 \pm 6.69$*** |
| 4f | 4-Cl | H | $23.34 \pm 1.71$** | $34.96 \pm 4.34$*** |
| 4g | 4-Cl | 4-Cl | $50.00 \pm 1.91$ | $56.61 \pm 6.06$* |
| 4h | 4-Cl | 4-CH$_3$ | **59.22 ± 2.33*** | **77.07 ± 5.61*** |
| 4i | 4-Cl | 4-OCH$_3$ | $30.00 \pm 4.50$*** | $43.84 \pm 2.60$*** |
| 4j | 4-Cl | 4-NO$_2$ | $12.24 \pm 3.71$** | $22.12 \pm 3.39$*** |
| Etoricoxib | | | **58.33 ± 3.67*** | **85.53 ± 1.63** |

*(n = 6), *** = p < 0.001, ** = p < 0.01, * = p < 0.05*

Gastric ulcerogenic effects were determined in rats [14] for representative examples of the synthesized compounds, 4c and 4h. Results indicated that compounds 4c and 4h did not induce any ulcerogenic effect at 50 mg/kg dose. At higher doses, the tested compounds exhibited low gastric ulcerogenicity compared with etoricoxib.

**Table 2 Ulcerogenic effects of compounds 4c and 4h in comparison with etoricoxib**

| Compounds | Dose mg/kg | Ratio of ulcered animals | Ulcer index (mean ± SEM) |
|---|---|---|---|
| Etoricoxib | 50 | 1/6 | $0.50 \pm 0.1$* |
| | 100 | 1/6 | $0.72 \pm 0.15$*** |
| 4c | 50 | 0/6 | 0.00 |
| | 100 | 1/6 | $0.35 \pm 0.23$* |
| 4h | 50 | 0/6 | 0.00 |
| | 100 | 1/6 | $0.75 \pm 0.20$*** |

*(n = 6), *** = p<0.001, ** = p<0.01, * = p<0.05*

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